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Impact of vitamin E or selenium deficiency on nematode-induced alterations in murine intestinal function ☆

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Abstract

The effects of deficiencies in the antioxidant nutrients, vitamin E and selenium, on the host response to gastrointestinal nematode infection are unknown. The aim of the study was to determine the effect of antioxidant deficiencies on nematode-induced alterations in intestinal function in mice. BALB/c mice were fed control diets or diets deficient in selenium or vitamin E and the response to a secondary challenge inoculation with *Heligmosomoides polygyrus* was determined. Egg and worm counts were assessed to determine host resistance. Sections of jejunum were mounted in Ussing chambers to measure changes in permeability, absorption, and secretion, or suspended in organ baths to determine smooth muscle contraction. Both selenium and vitamin E deficient diets reduced resistance to helminth infection. Vitamin E, but not selenium, deficiency prevented nematode-induced decreases in glucose absorption and hyper-contractility of smooth muscle. Thus, vitamin E status is an important factor in the physiological response to intestinal nematode infection and may contribute to antioxidant-dependent protective mechanisms in the small intestine.

Index Descriptors and Abbreviations: GI, gastrointestinal; *Hp*, *Heligmosomoides polygyrus*; 5-HT, 5-hydroxytryptamine; MO, menhaden oil; I_{sc}, short circuit current

1. Introduction

Nutritional status exerts an important influence on the immune system and is correlated with both the severity of and susceptibility to infection in nutritionally deprived populations in underdeveloped countries (Berkman et al., 2002; Sawaya et al., 1990). Specific dietary components, including the antioxidant nutrients vitamin E and selenium, have been identified as essential factors for normal immune function (Bauersachs et al., 1993; Beck et al., 1995; Forceville et al., 1998). Both infection and malnutrition are associated with increased formation of reactive oxygen species (Navarro et al., 1998). Vitamin E and selenium may be inadequately absorbed in gastrointestinal and liver diseases or have limited availability in patients who are critically ill or have inflammatory bowel disease (Forceville et al., 1998; Hinks et al., 1988). Selenium acts via glutathione peroxi-

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dase enzymes to protect lipid membranes from oxidant damage (Neve, 2000), while the fat-soluble vitamin E prevents propagation of free radical reactions (Demirel-Yilmaz et al., 1998; Navarro et al., 1998). Beck et al. (1995) showed that selenium and vitamin E deficiencies enhanced the virulence of Coxsackie virus and worsened virus-induced pathology in infected mice.

An estimated 3.5 billion people worldwide are affected by parasitic worm infection (Al-Shammari et al., 2001). In developing countries, enteric parasitic infections are a major cause of morbidity in childhood. There is strong evidence that poor nutritional status has a detrimental impact on the immune response to enteric parasite infection. *Heligmosomoides polygyrus* (*H. polygyrus*) is a gastrointestinal nematode parasite that naturally infects mice and is used to study immune response in the gut (Boulay et al., 1998; Morimoto et al., 2004; Shea-Donohue et al., 2001). Protein deficiencies promote survival of *H. polygyrus* by inhibiting intestinal production of Th2-mediated immunity (Ing et al., 2000). Helminth-induced alterations in intestinal epithelial and smooth muscle function are important in parasite expulsion (Donohue et al., 2001; Madden et al., 2002, 2004; Morimoto et al., 2004; Shea-Donohue et al., 2001). Enteric helminth infections like *H. polygyrus* elevate Th2 cytokines (interleukin-4 (IL-4) and -13 (IL-13)) and exert profound effects on intestinal epithelial cell (Colgan et al., 1994; Madden et al., 2004; Shea-Donohue et al., 2001) and smooth muscle function (Akiho et al., 2002; Zhao et al., 2003). Of interest is that these effects are similar to those exerted by IL-13 or IL-4 alone (Madden et al., 2002; Zhao et al., 2003). Moreover, both cytokine- and helminth-induced changes in intestinal function are not observed in mice deficient in STAT6, the signaling molecule for IL-4 and IL-13, which also contributes to resistance (Akiho et al., 2002; Madden et al., 2002; Zhao et al., 2003). Since Th2 cytokine-induced changes in intestinal physiology during *H. polygyrus* infection are linked to effective worm expulsion, the aim of the present study was to determine (1) the effect of diets deficient in the antioxidant nutrients, selenium or vitamin E, on intestinal function and (2) to determine the impact of these deficiencies on *H. polygyrus*-induced alterations in intestinal function and host resistance.

2. Methods

2.1. Diet

Female Balb/c mice (National Cancer Institute, Frederick, MD) were divided randomly into four diet groups ($n = 10$–14 each) that were fed ad lib for a total of 13–15 weeks after weaning. Mice received one of four torula yeast-based low selenium diets (prepared by Harlan Teklad, Madison, WI) that were adequate in all nutrients except those specified and are modifications of previously described diets (Beck et al., 1994). The two control diets were either an adequate diet with 4% lard/1% corn oil as the major fat source (Se+,VE+, and Lard) or an adequate diet with 4% menhaden oil (MO)/1% corn oil in place of lard as the fat source (Se+,VE+, and MO). Control adequate diets contained 0.2 μg/g sodium selenite and 50 mg/kg D-α-tochopherol acetate. The MO containing diet served as the control diet in experiments where vitamin E deficient diets were utilized. MO is known to increase the requirement for vitamin E and therefore, hastens the onset of a vitamin E deficiency (Dam, 1962). The two deficient diets used were either selenium deficient, lard-containing diet (Se−,VE+, and Lard) or vitamin E deficient MO-containing diet (Se+,VE−, and MO) where sodium selenite or D-α-tochopherol, respectively, were removed from the formulation.

2.2. Infection

Half of the mice in each dietary group ($n = 5$–7) received an oral inoculation of 200 infective, third stage *H. polygyrus* larvae (specimens on file at the US National Parasite Collection, US National Helminthological Collection, Beltsville, MD; Collection No. 81930) that were propagated and stored at 4 °C using a standard protocol (Madden et al., 2002, 2004; Shea-Donohue et al., 2001; Svetic et al., 1993; Zhao et al., 2003). Briefly, 14 days after the first inoculation, mice were treated with the anti-helminthic drug, pyrantel pamoate, to cure the infection. Uninfected control mice were treated with drug in an identical fashion. Thirty days later, mice were given a second inoculation of *H. polygyrus* and in vitro physiological measurements were performed 14 days later to coincide with the time of active expulsion in healthy mice (Urban et al., 1995). A secondary infection induces a more rapid IL-4 response and a greater IL-13 response than a primary infection (Finkelman et al., 2000) and with fewer changes in gut function (Shea-Donohue et al., 2001). To determine the effect of the diets on parasite fecundity and host resistance, quantitative fecal egg counts, and number of worms present in the intestine were determined at 30 after the second inoculation when worm expulsion is nearly complete (Smith et al., 2005). Fecundity was determined by dividing the total number of eggs recovered from the infected mouse by the total number of worms recovered to yield a total number of eggs produced per worm (Urban et al., 1995).

2.3. Ussing chambers

Four 1 cm segments of mucosa were stripped of muscle and mounted in Ussing chambers that exposed $0.126\,cm^2$ to 10 ml of Krebs' buffer. Agar-salt bridges and electrodes were used to measure potential difference. Every 50 s, the tissues were short-circuited at 1 V for 10 s

(World Precision Instruments DVC 1000 V clamp, Sarasota, FL) to allow calculation of tissue resistance using Ohm's law.

Following a 15 min equilibrium period, basal I_{sc}, representing the net ion flux at baseline, and tissue resistance, a measure of tissue permeability, were determined. After a second 15 min period, concentration-dependent changes in I_{sc} expressed in $\mu A/cm^2$ were determined in response to the cumulative addition of the secretagogues 5-hydroxytryptamine (5-HT, 10 nM–1 mM) or acetylcholine (1 nM–1 mM) to the serosal side of the stripped mucosae. After addition of the final concentration of each secretagogue, the Krebs' buffer on each side of the chamber was replaced and the tissue was allowed to equilibrate for 30 min. Upon re-equilibration, concentration-dependent changes in I_{sc} were measured in response to the cumulative addition of glucose (0–40 mM) to the mucosal side. Responses from all tissue segments exposed to glucose from an individual mouse were averaged to yield a mean response per animal.

2.4. Smooth muscle

Segments of jejunum (1 cm) were flushed of their intestinal contents and suspended longitudinally in organ baths. One end of the tissue was attached to an isometric tension transducer (Model FT03; Grass Medical Instruments, Quincy, MA, USA) and the other to the bottom of the bath. Tissues were stretched to a load of 9.9 mN (2 g). Preliminary experiments showed that this load stretched tissues to their optimal length for active contraction. Tissues were then allowed to equilibrate for at least 30–45 min in Krebs' buffer solution. The bath solution was replaced every 10 min throughout each study. Tension was recorded using a Grass model 79 polygraph (Grass Medical Instruments, Quincy, MA, USA) and expressed as force per cross sectional in mN/cm^2 (Zhao et al., 2003). Frequency-dependent responses to electrical field stimulation (EFS, 1, 2.5, 5, 10, and 20 Hz, 1 ms duration, 80 V) and concentration-dependent response curves to acetylcholine (1 nM–1 mM) were constructed in separate muscle strips.

2.5. Solutions and drugs

Krebs' solution contained (in mM) 118.5 NaCl, 4.75 KCl, 2.54 $CaCl_2$, 1.19 $MgSO_4$, 25.0 $NaHCO_3$, 1.19 NaH_2PO_4, and 11.0 dextrose. For the Ussing chambers, the tissues were exposed to 11 mM glucose on the serosal side and 10 mM mannitol on the mucosal side. Mannitol, a non-absorbable sugar, is used to balance the osmotic effects of the glucose placed on the serosal side. All drugs were obtained from Sigma (St. Louis, MO) unless stated otherwise. Stock solutions of acetylcholine (1 μM) were prepared in ultrapure water and frozen. On the day of the experiment, 5-HT was dissolved in water and appropriate dilutions of acetylcholine, 5-HT, and glucose were made using distilled water.

2.6. Histology

Full thickness sections of mid jejunum from each animal were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with Giemsa. Using a previously validated scoring system for mice (Fleming et al., 2003), microscopic changes in morphology were assessed by two independent investigators who were unaware of the treatment group. Photomicrographs were taken using a Nikon E800 (Nikon, Melville, NY).

2.7. Data analysis

Statistical analysis was performed using one-way ANOVA to assess changes in worm and egg production or tissue resistance followed by a Neuman–Keul's *t* test to compare difference among multiple means. Cumulative concentration responses were compared using MANOVA with post hoc analysis for multiple comparisons. A $p < 0.05$ was considered significant.

3. Results

3.1. Host resistance

The number of eggs in the feces and adult worms present in the intestinal lumen were used as indices of parasite fecundity and host resistance, respectively (Urban et al., 1995). As demonstrated previously (Smith et al., 2005), there is active worm expulsion starting around 14 days after a second inoculation of *H. polygyrus* that becomes more effective over time with nearly complete worm expulsion by day 30 after challenge in selenium and vitamin E adequate control mice (Se+/VE+). In contrast, mice fed diets deficient in either selenium (Se−/VE+) or vitamin E (Se+/VE−) had a greater worm burden and increased number of eggs indicating a reduced resistance *H. polygyrus* (Table 1).

3.2. Histology

There were no differences in morphology of the small intestine in uninfected mice fed the four individual diets. *H. polygyrus* infection induced moderate cellular infiltration in and around cysts in the submucosa of the duodenum (Morimoto et al., 2004) and an increase in goblet cell mucus, but had little effect on overall epithelial cell morphology. There were no differences in the morphological appearance of the mucosa among any of the groups of infected mice on the various diets (data not shown).

Table 1
The effect of the selenium (Se) and vitamin E (VE) deficient diets on worm and egg counts obtained 30 days after a secondary infection with *H. polygyrus*

	Diet			
	Se+/VE+ Lard	Se−/VE+ Lard	Se+/VE+ MO	Se+/VE− MO
Adult worms	2 ± 1	$56 \pm 11^{*}$	3 ± 2	$39 \pm 19^{*}$
Fecal eggs	114 ± 114	$9991 \pm 2709^{*}$	75 ± 41	$10075 \pm 5632^{*}$
Eggs/worm	57 ± 30	$143 \pm 15^{*}$	53 ± 27	$142 \pm 43^{*}$

Values are means ± SEM; $^{*}p < 0.05$ vs respective control diet; and $n \geqslant 5$ in each group.

3.3. Effect of diets on epithelial cell responses

There were few differences among the individual diets in uninfected mice in most of the key parameters of epithelial cell function. The only diet-related difference was that the maximal response to acetylcholine was elevated in the uninfected mice fed the Se+/VE− diet (Table 2). Infection reduced secretion in response to 5-HT (Tables 2 and 3) or acetylcholine (Tables 2 and 3) regardless of diet. In mice fed the replete diets, *H. polygyrus* infection decreased mucosal resistance (Fig. 1A and Table 3) and inhibited sodium-linked glucose absorption (Fig. 1B and

Table 2
Effects of the vitamin E (VE) deficient diet on maximal secretory responses to acetylcholine (Ach) and 5-HT in uninfected and *H. polygyrus*-infected mice

	Diet			
	Se+/VE+	*Hp* Se+/VE+	Se+/VE−	*Hp* Se+/VE−
ACH (1 mM)	108 ± 9	$48 \pm 17^{*}$	$191 \pm 31^{**}$	$54 \pm 18^{*}$
5-HT (100 μM)	42 ± 4	$12 \pm 6^{*}$	67 ± 28	$4 \pm 2^{*}$

Values are maximal responses expressed in $\mu A/cm^2$; means ± SEM; $^{*}p < 0.05$ vs respective uninfected group; $^{**}p < 0.05$ vs control diet; $n \geqslant 4$ in each group; and all diets contain MO.

Table 3
Effects of the selenium (Se) deficient diet on epithelial cell function in uninfected and *H. polygyrus*-infected mice

	Diet			
	Se+/VE+	*Hp* Se+/VE+	Se−/VE+	*Hp* Se−/VE+
ACH (1 mM)	128 ± 28	79 ± 33	115 ± 8	$48 \pm 13^{*}$
5-HT (100 μM)	29 ± 10	$11 \pm 6^{*}$	15 ± 4	$7 \pm 1^{*}$
Glucose (40 mM)	332 ± 20	$77 \pm 32^{*}$	220 ± 49	$101 \pm 25^{*}$
R ($\Omega\,cm^2$)	21 ± 1	$10 \pm 1^{*}$	24 ± 6	$13 \pm 2^{*}$

Values are maximal responses expressed in $\mu A/cm^2$; $^{*}p < 0.05$ vs respective uninfected group; means ± SEM; $n \geqslant 3$ in each group; and all diets contain Lard.

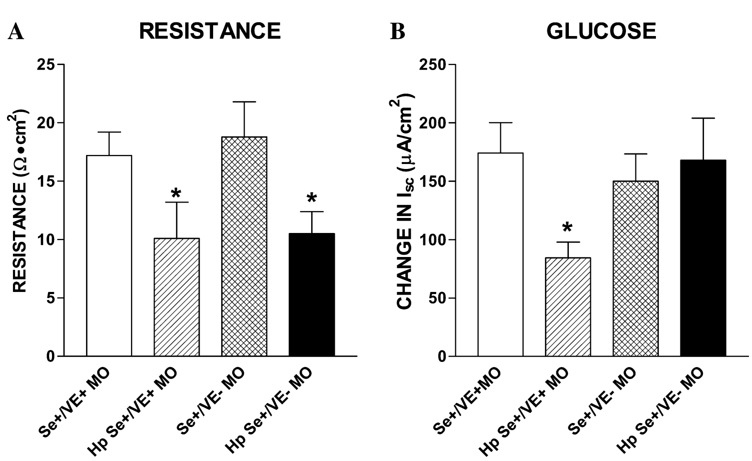

Fig. 1. Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure changes in tissue resistance (A, an index of epithelial permeability) or sodium-linked glucose absorption (B) in BALB/c uninfected controls or *H. polygyrus*-infected mice. Control and infected mice were fed diets replete or deficient in vitamin E. All diets contain menhaden oil (MO). The responses to glucose are maximal responses to 40 mM. Values are means ± SE; * $p < 0.05$ vs Se+/VE+ MO diet; $n \geqslant 4$ in each group.

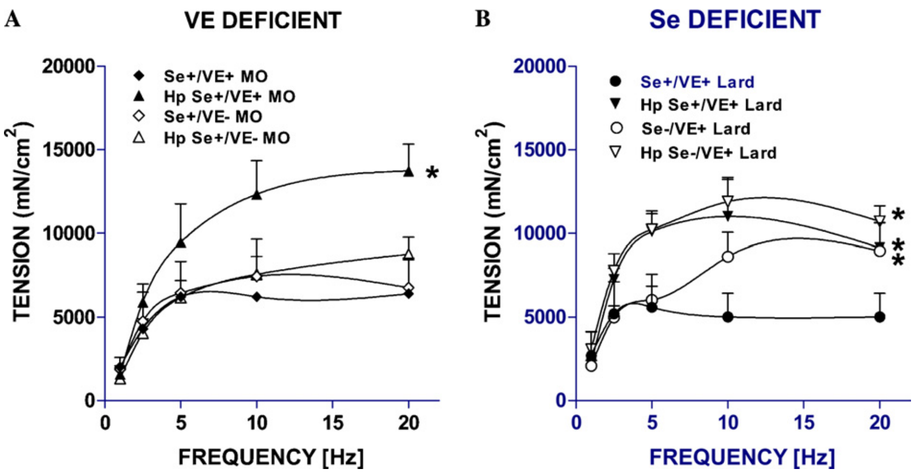

Fig. 2. Frequency-dependent responses to electrical field stimulation of intestinal longitudinal smooth muscle in BALB/c uninfected controls or *H. polygyrus*-infected mice. Control and infected mice were fed diets replete or deficient vitamin E (VE) (A) or selenium (Se) (B). Control diets contain menhaden oil (MO) or lard, respectively. Segments of jejunum were suspended longitudinally in organ baths and subjected to electrical field stimulation (1–20 Hz, 80 V, 1 ms). Values are means ± SE; $p < 0.05$ vs appropriate Se+/VE+ diet; $n \geqslant 5$ in each group.

Table 3). These effects are consistent with previous studies of *H. polygyrus* (Morimoto et al., 2004; Shea-Donohue et al., 2001). In mice fed the Se−/VE+ diet, *H. polygyrus*-induced alterations in resistance or glucose absorption (Table 3) were not significantly different from those in mice fed the replete diet, demonstrating that delayed worm expulsion occurs in selenium deficiency despite functional changes that increase intraluminal fluid. In contrast, in mice fed the Se+/VE− diet, the infection-induced reduction in glucose absorption was not observed (Fig. 1B) and coincided with the impaired worm clearance (Table 1). *H. polygyrus*-induced alterations in resistance, however, were similar to mice on the replete diet (Fig. 1A).

3.4. Effect of diets on smooth muscle responses

In uninfected mice, the different diets had no effect on smooth muscle responses to acetylcholine (Fig. 3); however, responses to EFS were elevated in uninfected mice fed the Se−/VE+ diet (Fig. 2). Consistent with previous data (Zhao et al., 2003), expulsion of *H. polygyrus* infection is associated with a hyper-contractility of smooth muscle to nerve stimulation (Fig. 2B) and acetylcholine (Fig. 3B) in mice fed the replete diets. Infection-induced hyper-contractility of smooth muscle responses to EFS (Fig. 2A) or to acetylcholine (Fig. 3A) were intact in mice fed the Se−/VE+ diet indicating that worm expulsion was delayed despite increased

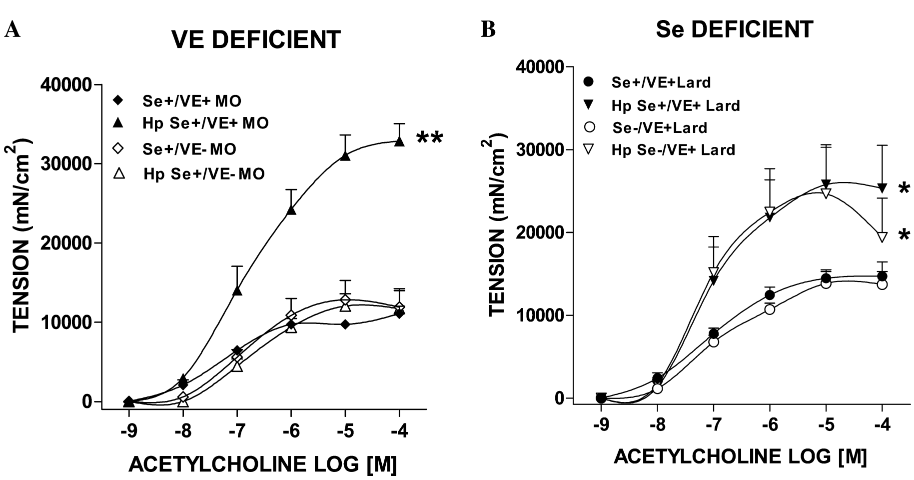

Fig. 3. Concentration-dependent responses to acetylcholine of intestinal longitudinal smooth muscle in BALB/c uninfected controls or *H. polygyrus*-infected mice. Control and infected mice were fed diets replete or deficient vitamin E (VE) (A) or selenium (Se) (B). Control diets contain menhaden oil (MO) or lard, respectively. Segments of jejunum were suspended longitudinally in organ baths and challenged with acetylcholine (1 nM–1 μM). Values are means ± SE; $p < 0.05$ vs appropriate Se+/VE+ diet; $n \geqslant 5$ in each group.

smooth muscle contraction. In contrast, in mice fed the Se+/VE− diet, delayed expulsion was associated with an inhibition of *H. polygyrus*-induced hyper-contractility to both nerve stimulation (Fig. 2A) and acetylcholine (Fig. 3A).

4. Discussion

Expulsion of helminth parasites requires a coordination of both immune and functional host responses (Palmer and Greenwood-Van Meerveld, 2001). There are a number of factors that can influence host resistance to infection including nutritional status. Recognition of this interaction underlies public health concerns about malnutrition as a risk factor for infectious diseases (Scrimshaw, 2003). It also promotes the concept of nutrient-directed management of immune-based pathologies (Field et al., 2002) and stimulates use of functional foods that deliver specific nutrients that target the immune system (Calder and Kew, 2002). There are studies showing the benefits of supplementation of the antioxidant nutrients, vitamin E, and selenium, on host defense (Batra et al., 1993; Daoud et al., 2000; McCarthy and Davis, 2003), but there is little information of impact of deficiencies in these nutrients on host resistance to infection.

Effective clearance of gastrointestinal nematodes is dependent on the induction of Th2 cytokines and a dampened IFN-γ response (Urban et al., 2000; Urban et al., 1995). IL-4 is integral to host defense resulting in reduced worm fecundity and adult worm survival and induces significant alterations in intestinal function that facilitate worm expulsion (Madden et al., 2004; Shea-Donohue et al., 2001; Urban et al., 2000). This hypothesis is supported by studies showing that Th2 and nematode-induced changes are attenuated or absent in mice lacking STAT6 (Madden et al., 2002, 2004; Zhao et al., 2003), the nuclear signaling factor activated by receptor binding of IL-4 or IL-13. Nematodes elicit a stereotypic IL-4-induced and STAT6-dependent increase in intestinal permeability, a decrease in sodium-linked glucose absorption, and an increase in smooth muscle response to acetylcholine and nerve stimulation. These effects are temporally correlated with active worm expulsion (Madden et al., 2002; Shea-Donohue et al., 2001; Zhao et al., 2003).

In the present study, we demonstrated that mice fed diets deficient in vitamin E alone had little impact on mucosal morphology in *H. polygyrus* infected mice. The selenium deficient diet significantly increased epithelial cell response to acetylcholine and elevated contractions to EFS, but the majority of functional parameters measured were unchanged. When immune challenged, however, the mice fed the deficient diets exhibited impaired host resistance to *H. polygyrus* infection as evidenced by a prolonged delay in egg and worm clearance even in the presence of elevated levels of circulating IL-4 (Smith et al., 2005). The impaired worm expulsion and increased number of eggs apparent in infected mice fed diets deficient in either vitamin E or selenium, therefore, could not be attributed to an inappropriate cytokine response or to secondary morphological changes affecting the epithelial cell barrier. The impact of deficiencies in selenium or vitamin E alone on intestinal function is unknown; therefore, we first investigated epithelial cell and smooth muscle function in healthy mice fed replete or deficient diets. Selenium deficiency alone increased response to EFS while vitamin E deficiency elevated responses to ACH, consistent with previous results in small intestine of rat deprived of vitamin E (Lindley et al., 1993). Deficiencies in these dietary factors alone, therefore, do not appear to have major consequences on the physiological regulation of intestinal function. Because nutritional status is generally considered to be a critical factor in modulating the host response to immune stressors such as enteric parasite infection, we investigated the impact of selenium or vitamin E deficiencies on the host response to a secondary *H. polygyrus* infection. Gastrointestinal nematode parasites induce an increase in intraluminal fluid and a hyper-contractility of smooth muscle as part of a “weep and sweep” response that facilitates worm expulsion (Madden et al., 2002; Armstrong, 1987). Mice deficient in selenium or in vitamin E had impaired functional responses to *H. polygyrus* resulting in delayed expulsion at 30 days.

To investigate further the mechanism involved in the impaired worm expulsion observed in both selenium and vitamin E deficiencies, we evaluated the effects of the deficiencies on infection-induced alterations in intestinal mucosal and smooth muscle function. Surprisingly, there were marked differences in the effects of these dietary deficiencies on several critical functional parameters involved in worm clearance. Mice fed the vitamin E deficient diet exhibited selective permutations in intestinal function following infection. The expected *H. polygyrus*-induced inhibition of glucose absorption and increase in smooth muscle contractility in response to nerve stimulation or acetylcholine were not observed in infected mice fed the vitamin E deficient diet. The enhanced mucosal permeability and antisecretory effects in response to acetylcholine or 5-HT, however, remained intact. One possible mechanism for the change in glucose absorption could be alterations in sodium-linked glucose transporter (SGLT1) on the brush border (Armstrong, 1987). There is considerable “plasticity” of the intestinal epithelial cell lining such that the expression of nutrient transporters is influenced likely by changes in caloric restriction or parasite infection. Ferraris et al. (2001) showed that chronic caloric restriction to 70% of caloric needs did not change intestinal mass, and yet, increased glucose, fructose, and proline absorption after 270 days of restricted diet. In the present study, the delayed worm clearance and increased fecal egg count in the infected group fed the vitamin E deficient diet supports the concept that intraluminal fluid, resulting from decreased glucose absorption, and hyper-contractility of smooth muscle, contribute to host resistance.

In contrast to the results observed in vitamin E deficient infected mice, there were few differences in *H. polygyrus*-induced changes intestinal function between diets replete or deficient in selenium. Although selenium deficiency alone induced a hyper-contractility to EFS that was not increased further by infection, the hyper-contractile responses to acetylcholine were observed in mice fed selenium deficient or replete diets. These effects suggest that increased intraluminal fluid and hyper-contractility of smooth muscle are not necessarily sufficient to induce worm expulsion and other factors in addition to upregulation of Th2 cytokines and alterations in intestinal function are important for worm expulsion. These data suggest that effective worm expulsion is a complex multifaceted process. The mechanism underlying the delayed worm expulsion in selenium deficient mice remains unclear, but does not appear to involve effects on the worm per se, since both fecundity and number of worms were similar in each group. One factor may be the duration of the functional responses. Although not tested directly in this study, changes in intestinal function are evident prior to expulsion and continue for a period afterwards. Selenium deficiency may truncate the duration of this interval with the expectation of an increasing disparity in worm clearance between replete and deficient groups over time. Alternately, there may be differences in the local cytokine responses between the vitamin E and selenium deficient diets that do not parallel changes in circulating levels.

Other studies have demonstrated that caloric deficiency negatively impacted host resistance to helminth infections as evidenced by delayed worm expulsion and a prolonged survival of the parasite (Boulay et al., 1998). Malnourished, parasite-infected mice had smaller body mass, enhanced glucose transport activity, and lower resting metabolism compared to control mice (Kristan and Hammond, 2001). The consequences of chronic infection or multiple co-infections are aggravated by malnutrition usually as a result of chronic nutrient malabsorption caused by parasite injury to the intestinal mucosa (Al-Shammari et al., 2001; Tshikuka et al., 1997). These effects were associated with poor upregulation of Th2 cytokines or mucosal damage that underlies the impaired expulsion. In contrast, there is a normal elevation in IL-4 production in mice on the deficient diets (Smith et al., 2005) and we show here that the deficient diets did not alter mucosal morphology. Indeed, decreased tissue resistance during infection with *H. polygyrus* is IL-4 and Stat6-dependent (Madden et al., 2002; Shea-Donohue et al., 2001) and is comparable in infected mice regardless of the diet suggesting that functional levels of IL-4 were available to the intestinal epithelium and that any vitamin E or selenium-dependent responses are down stream of IL-4 activation.

In conclusion, these data demonstrate that dietary deficiencies alone have little impact on physiological control of normal intestinal function, but significantly impact specific immune-mediated changes necessary for host resistance to *H. polygyrus* infections. Selenium and vitamin E deficiency impaired expulsion of *H. polygyrus* despite upregulation of IL-4 and IL-4-dependent changes in tissue resistance. The reduced host resistance in the vitamin E deficient mice could be attributed directly to the absence of key intestinal epithelial cell and smooth muscle functions that normally facilitate worm clearance expulsion. *H. polygyrus*-induced changes in intestinal function were intact in selenium deficient mice suggesting the involvement of other mechanisms. Vitamin E appears to be a critical factor in the functional response to enteric infection and deficiency may contribute to the chronicity of infection in prevalent areas.

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